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Exercise-Induced Changes in Neurotrophic Factors and Markers of Blood-Brain Barrier Permeability Are Moderated by Weight Status in Multiple Sclerosis

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ABSTRACT

Blood-brain barrier (BBB) and neurotrophic factors seemingly have an important role in multiple sclerosis pathology. Physical activity may influence blood-brain barrier function and levels of neurotrophic factors, and such effects might be moderated by body weight status. This study investigated the effect of exercise training on markers of blood-brain barrier permeability and neurotrophic factors as a function of weight status in multiple sclerosis patients. Sixty three persons with relapsing remitting multiple sclerosis who were normal weight (n: 33) or overweight (n: 33) were randomly assigned into groups of exercise (normal weight training, n: 18; overweight training group, n: 18) or no exercise (normal weight control, n: 15; overweight control group, n: 15). The intervention consisted of 8 weeks (3 days per week) of cycling undertaken at 60-70% peak power. Resting blood concentrations of s100 calcium-binding protein B (s100b) and neuron-specific enolase as BBB permeability markers, neurotrophic factors and cytokines (Interleukin-10 and tumor necrosis factor alpha) were evaluated before and after the intervention. There were significant weight, training, and interaction effects on brain-derived neurotrophic factor and platelet-derived growth factor; however, ciliary neurotrophic factor and nerve growth factor did not demonstrate any effect. Brain-derived neurotrophic factor and platelet-derived growth factor were significantly increased from pre-post in normal weight exercise. Significant weight, training, and interaction effects were found for s100b. In detail, s100b was significantly increased from pre-post in normal weight exercise. In contrast, neuron-specific enolase and cytokines did not demonstrate any effect. Generally, Exercise training may alter markers of BBB permeability and neurotrophic factor status in normal weight persons with multiple sclerosis; however, overweight participants may be more resistant to these effects of exercise.

Keywords: Blood-brain barrier, Neurotrophic factors, Cytokine, Exercise, Excess weight, Multiple sclerosis

1. Introduction

Multiple sclerosis is a common and unpredictable neurological disease of the central nervous system (CNS) (Mokhtarzade et al., 2017). The cause of multiple sclerosis is currently unknown, but seemingly involves an environmental trigger of the immune system that results in demyelination and impaired remyelination processes with the CNS of genetically susceptible persons (Motl and Pilutti, 2012; Motl et al., 2017). Such an immune-mediated process involves disruption of the blood-brain barrier (BBB)(Roh et al., 2016) as well as a lack of neurotrophic support for repair (Bansi et al., 2013; Wens et al., 2016; Zimmer et al., 2017). The outcome of CNS demyelination is associated with impaired nervous conduction and reduction in motoric and cognitive abilities (Mokhtarzade et al., 2017; Solari, 2015).

The BBB monitors the entry of the essential and useful substance into the CNS, as well as restricting the passage of harmful substances (Ballabh et al., 2004; Obermeier et al., 2013; Roh et al., 2016). BBB dysfunction, either acute or chronic, results in the entry of harmful substances such as toxic metabolites, glucocorticoids (e.g. cortisol), pro-inflammatory cytokines (e.g. tumor necrosis factor alpha; TNFα), and immune cells into the CNS (Ortiz et al., 2014). This is seemingly a critical process in the pathophysiology of multiple sclerosis, as auto-reactive immune cells can enter the CNS during periods of BBB dysfunction and initiate the cascade of events resulting in demyelination and impaired repair (Larochelle et al., 2011; Ortiz et al., 2014). There are several methods of measuring BBB permeability and function (Côté et al., 2010; Montagne et al., 2015). A valid method that does not require magnetic resonance imaging (MRI), contrast agent injection, or cerebrospinal fluid (CSF) sampling is the monitoring of peripheral blood circulation markers of BBB function such as S100 calcium-binding protein B (s100b) and neuron-specific enolase (NSE) (Hoffmann et al., 2017; Roh et al., 2016). This issue is not limited to BBB markers. Most techniques for monitoring immune markers and neurotrophic factors in the CNS require CSF sampling; it can be helpful, therefore to use CNS-related peripheral markers instead of CSF sampling.

Regular physical activity, as a complementary therapeutic method (Mokhtarzade et al., 2017), that has been proposed as a putative disease modifying behavior in multiple sclerosis. A vast body of literature suggests positive effects of exercise on functional capacity, symptoms, and quality of

life outcomes (Motl et al., 2017) in pwMS. However, substantially less is known about its effect on multiple sclerosis pathophysiology including immune cells, cytokines, BBB function and neurotrophic factors (Motl et al., 2017; Wens et al., 2016; Zimmer et al., 2017). One of the reasons that may explain the uncertainty of the exercise effect on immune markers and neurotrophic factors is that exercise effects might depend on BBB permeability and/or weight status (Poduslo and Curran, 1996; Stranahan et al., 2009). Nevertheless, studies regarding the effect of exercise training on BBB permeability and related blood neurotrophic factors have not been conducted in overweight or obese multiple sclerosis patients.

We conducted a randomized control trial (RCT) examining the effect of aerobic exercise training on BBB permeability markers, neurotrophic factors and cytokines, and further examined if weight status moderated the effect of exercise on these parameters. We hypothesized that exercise training vs. control would improve markers of BBB permeability, neurotrophic factors and cytokine profiles. We further hypothesized that weight status may affect markers of BBB permeability and neurotrophic factors following exercise training as well as cytokine status. To test this hypothesis, we recruited overweight and normal weight persons with multiple sclerosis and investigated the effect of interval exercise training on BBB permeability, cytokine profile, and neurotrophic factors in these persons compared with a non-exercise control condition.

2. Materials and methods

2.1. Participants

The present study was approved by the Ethics Committee of Islamic Azad University of Abadan, Abadan, Iran and was performed in accordance with the Declaration of Helsinki. The full trial was conducted between Nov 2015 and May 2017. Participants with relapsing-remitting multiple sclerosis were recruited from Khuzestan multiple sclerosis center, Ahvaz, Iran. There were 108 relapsing-remitting multiple sclerosis patients with age > 22 years expressing an interest in participating in current study who were screened for inclusion criteria. All risks and

benefits of the present study were explained to participants, and then written informed consent was obtained.

As illustrated in Figure 1, 103 of the 108 multiple sclerosis patients (Expanded Disability Status Scale; EDSS ≤ 4, age > 22 year) were eligible based on inclusion/exclusion criteria (8 did not meet inclusion/exclusion criteria). Subjects were excluded if they participated in another study or had: a) change immune modulatory therapy within the last six months; b) engaged in physical activity more than two hours per week; c) a relapse or acute multiple sclerosis exacerbation within the last four months; d) smoked; e) other chronic diseases (metabolic, cardiovascular, kidney or renal); and f) particular diet such as vegetarian.

Of the 103 eligible participants, 54 participants had normal body weight status and 49 participants were considered overweight based on BMI. We then randomly selected 66 participants for participation, and 33 participants had normal weight status and 33 participants were overweight. These participants were randomly divided into exercise or control groups with a gender ratio of two:one (female:male) per group. Therefore, both the exercise and control groups included two subgroups of overweight and normal weight status. The groups included overweight training group (OW-trained, n=18), normal training group (NW- trained, n=18), overweight control group (OW- control, n=15), and normal control group (NW-control, n=15). During the intervention program one subject was lost to follow up from the NW- training group due to a relapse; one subject from the NW-control and the OW-trained group; and two subjects from the OW-control group were also lost to follow up due to being unwilling to continue in the study. Participant flow through recruitment/enrollment is included in the Consolidated Standards of Reporting Trials diagram (Figure 1).

2.2. Procedures

Pre-test data were collected from subjects before starting the exercise program. Pre-test data was also collected at the same time from the control group. Four days before starting the study, a venous blood sample was taken from the subjects in a seated position after 10 min rest. Next day, subjects performed a peak oxygen uptake (VO_{2peak}) test as described previously (Storer et al., 1990). Although the assessors were blinded, based on nature of the study, neither therapy

administrations nor subjects were blinded to group allocation. Furthermore, although both OW-trained and NW-trained groups were enrolled in the 8-week interval exercise training group, neither of the OW-control and NW-control groups participated in any exercise training. After completing the training program or control period, all pre-test measures were repeated. The blood samples were taken two days after completing exercise training. Finally, the VO_{2peak} test was performed three days after completing the training program.

2.3. Training program

The training program consisted of 8 weeks (three sessions per week) of upper- and lower-body interval-training using a Monark 891E and 894E ergometers (Varberg, Sweden), respectively. Each session consisted of three phases. Firstly, subjects performed stretching and low-intensity cycling as a warm-up protocol (ten min). Secondly, the study participants completed the main body of the program consisting of three intervals of upper and lower extremity cycling. After completion of the interval training, stretching exercises were performed as a cool-down (ten min). The main body of the exercise program consisted of three intervals (two min active and two min inactive rest) in week one and gradually increased to six intervals in week 8. It should be noted that intervals were considered separately for upper and lower limbs. The training load was adjusted by watt peak (W_{peak}) determined by the VO_{2peak} test. Training load started at 60% W_{peak} during the first week and increased 5% W_{peak} for each two weeks and eventually reached 75% W_{peak} in the eighth week. During all sessions, the pedal rate was fixed at 50 rpm. The lower and upper limbs W_{peak} was determined as described previously (Sawka et al., 1983; Storer et al., 1990). During all training sessions, heart rate was recorded and found to be between 60% and 80% maximal heart rate in active intervals. The session duration was incremental between 42 and 66 min (supplementary materials Figure 1).

The control groups did not participate in any regular exercise program and were instructed to continue routine exercise and lifestyle behaviors. The control groups only completed pre-post outcome assessments. This condition was selected as a control for passage of time, maturation, and testing as confounders toward the internal validity of our experiment, but it did not account for social contact and attention as other confounders toward internal validity. A control group is

appropriate for this early stage of research, but future research might consider a minimal exercise condition such as supervised stretching for controlling other possible influences on study outcomes.

2.4. Body mass index

In this study, overweight body status is referred to as a person with a body mass index (BMI) above 25 kg/m^2. Body weight, height, and BMI were measured before and after the intervention period in 10-h fasting. BMI (kg/m^2) derived from the body mass (kg, Seca515, Sweden) divided by the square of height (m) of an individual.

2.5. Neurotrophic factors and cytokines assessment

Venous blood samples were collected in a clotted blood tube (BD Vacutainer Plus SST) before and after the eight-week training intervention after 10-h of fasting (between 8 and 9 a.m.). Subsequently, samples were centrifuged (3000 *g* for 12 min at 4 °C). Aliquots of serum were stored in Eppendorf tubes at -80℃ until analysis. Serum level of brain-derived neurotrophic factor (BDNF), nerve growth factor (NGF) was determined by enzyme-linked immunosorbent assay (ELISA) kits according to the manufacturer's instructions (R&D Systems, Minneapolis, USA). The serum level of ciliary neurotrophic factor (CNTF), and platelet-derived growth factor (PDGF) was determined by IBL International GMBH kit (Hamburg, Germany) according to the manufacturer's instructions. To determine serum level of cytokines (TNFα and interleukin 10; IL-10), IBL International GMBH kit (Hamburg, Germany) was used according to the manufacturer's instructions. A microplate reader (Biochrom, Cambridge, UK) was used to measure absorbance at 450 nm for quantification. Neurotrophic factors and cytokines coefficients of variation (CVs) were less than 8%. Serum level of cortisol was calculated by DiaMetra ELISA kit (Milano, Italy) with a CV equal to 6.5%.

2.6. BBB permeability markers

To illustrate BBB permeability status, s100b and NSE were used. Serum levels of s100b reflected BBB permeability and deterioration and therefore may predict brain injury severity (Roh et al., 2016; Roh et al., 2017). In addition, NSE is reflective of neuron damage, neuron destruction, ischemic damage, and structural impairments in neuronal cell walls (Marchi et al., 2004; Roh et al., 2017). In numerous studies, s100b is considered as the best indicator for BBB permeability and deterioration (Koh and Lee, 2014; Marchi et al., 2004; Roh et al., 2017), however, NSE often is considered as neuronal, but is often used as a nonspecific marker of BBB permeability (Marchi et al., 2004; Marchi et al., 2003; Roh et al., 2017). In the present study, the serum levels of s100b and NSE were determined by ELISA kit (Abnova, Taiwan and Alpha diagnostic, USA, respectively) and CVs were less than 8%.

2.7. Data analysis

Statistical analyses were performed using SPSS Statistics 21. After creating change scores for study outcomes based on the difference between pre- and post-test scores (post-test subtraction from pre-test), two-factor ANOVA (weight status: 2 levels of normal weight and overweight, conditions: 2 levels of exercise training and control) was used to investigate the effect of exercise training and weight status on changes of neurotrophic factors, BBB permeability, and cytokines or baseline levels of these factors. Partial Eta squared (η_p^2) was used to determine effect size in ANOVA tests. The Bonferroni test was applied for all analyses that needed a post-hoc test. An alpha level of 0.05 was considered to be statistically significant ($P \leq 0.05$).

3. Results

3.1. Baseline characteristics

There were no significant differences between conditions in the baseline demographic characteristics including age, EDSS and disease duration (P>0.05). VO_{2peak} was significantly higher in normal weight subjects when compared with overweight subjects (P<0.05, Table 1).

Baseline neurotrophic analysis indicated that only BDNF showed a significant difference (P<0.05), however, no significant difference was observed for any other neurotrophic factors (P>0.05). Furthermore, NSE data were significantly higher in overweight subjects (P<0.05) while there was no baseline difference for cytokine profile (P>0.05, Table 2).

3.2. Effect of exercise training on neurotrophic factors and BBB permeability

The two-factor ANOVA test indicated that BDNF changes had significant weight (95% Confidence Interval (CI)= -4.3 to 0.5 ng/ml, η_p^2: 0.394), training (95% CI= -2.4to 2.1 ng/ml, η_p^2: 0.543), and interaction effects (95% CI= 10.4 to 16.9 ng/ml, η_p^2: 0.556). Post-hoc analysis indicated that BDNF increased significantly in NW-trained compared with other groups (Figure 2A). There were no effects or interactions on NGF and CNTF (Figure 2B and C). There were significant weight (95% CI= -76.8 to 39.4 pg/ml, η_p^2: 0.74), training (95% CI= 31.4 to 142.6 pg/ml, η_p^2: 0.541), and interaction (95% CI= 42.4 to 198.2 ng/ml, η_p^2: 0.144) effects for PDGF changes. Both NW-trained and OW-trained had significant increases in PDGF compared with the control groups, and NW-trained had significantly higher increasing than OW-trained (Figure 2D, supplementary materials Table 2).

S100b changes, had significant weight (95% CI= -8.3- to 4.8 pg/l, η_p^2: 0.123), training (95% CI= -12.7 to -0.07pg/l, η_p^2: 0.297), and interaction (95% CI= -18.8 to -0.10 pg/l, η_p^2: 0.067) effects (Figure 2E). On the other hand, NSE changes indicated no significant effect (Figure 2F). Post-hoc analysis for s100b demonstrated that s100b decreased significantly more in NW-trained rather than other groups.

3.3. Effect of exercise training on cytokines profile, cortisol and Vo_{2peak}

TNFα (95% CI= -0.4 to 0.19 pg /ml, η_p^2: 0.061) demonstrated a significant weight status effect. This was demonstrated by the normal weight group experiencing more decrease compared to the overweight group. IL-10 and cortisol did not demonstrate any weight effect (Figure 3). In the current study, IL-10/TNFα ratio was used as a parameter reflecting anti-inflammatory status (Mokhtarzade et al., 2017). We observed that the IL-10/TNFα ratio did not demonstrate any significant main effects or interaction. On the other hand, none of the mentioned factors had any training and/or interaction effect. VO_{2peak} only indicated a training effect (Figure 4). The VO_{2peak} increase was significantly higher in NW-trained and OW-trained compared to NW-control and OW-control groups. Finally, VO_{2peak} remained significantly higher after the training program (supplementary materials Table 1).

4. Discussion

This is the first study investigating the physiological response of MS relevant biomarkers depending on subjects` weight status. We assumed that weight classification can be considered as a moderating factor for the effects of exercise on BBB function and neurotrophins in people with MS. Therefore, we conducted a RCT that examined the effects of 8 weeks aerobic interval exercise training on changes in disease modifying proteins including neurtotrophic factors (BDNF, NGF, CNTF and PDGF), cytokines (TNFα and IL-10), cortisol and BBB function markers (s100b and NSE) associated with MS between groups who differed in weight status (normal weight and overweight). To examine our hypothesis, 33 overweight patients and 33 normal weight patients were recruited and assigned into two conditions of exercise and control. Overall, exercise resulted in an increase of the neurotrophic factors, especially BDNF and PDGF depending on weight status; the highest increase in the levels of these two factors was observed in the normal weight participants who were involved in the exercise program. We further observed the effects of exercise on BBB permeability markers (S100b and NSE) indicating that participants with normal weight may be more adaptable for improving BBB permeability markers, especially s100b, to exercise training compared to overweight participants.

4.1. Exercise-Related Neurotrophic Factors Modulation

Results suggested that exercise training may enhance circulating levels of BDNF and PDGF; however, body weight influenced the effect of exercise on these two neurotrophic factors. The results of our study suggest that overweight participants had a blunted increase in BDNF and PDGF following exercise training compared to normal weight participants. Moreover, exercise training had no significant effect on CNTF and NGF. Previous studies strongly confirm that BDNF is a critical factor in myelin repair, and positively affects neuronal and axonal survival (Arora, 2006; Castellano et al., 2007; Onuoha et al., 1998). Therefore, upregulation of BDNF and increased serum levels of this factor can be a positive indicator for the role of exercise training in people with multiple sclerosis (Bansi et al., 2013; Castellano et al., 2007). Our findings are consistent with previous studies. For instance, Wens et al., and Bansi et al., reported that resistance training and aquatic training improve BDNF concentration in multiple sclerosis subjects, respectively (Bansi et al., 2013; Wens et al., 2016). Taken together, the few existing studies are in line with the present study, but our study expands the current knowledge by investigating the effect of body weight on neurotropic factors in multiple sclerosis.

Weight gain is associated with releasing leptin from adipose tissue which stimulates pro-inflammatory cytokines produced from T helper 1 cells such as TNFα (Majdinasab et al., 2018; Mokhtarzade et al., 2017). In addition, there are more macrophages, T cells, and inflammatory molecules connected with more subcutaneous adipose tissues (Esser et al., 2014). Therefore, obesity and multiple sclerosis are both associated with chronic inflammation that can together increase the severity of these conditions. Therefore, it can be concluded that the inflammation in overweight subjects observed in the previous studies can be attributed to lower efficacy of exercise training on neurotrophic factors (Roh et al., 2016). However, we observed that TNFα and IL-10 concentrations and IL-10/TNFα ratio did not statistically differ between normal weight and overweight participants.

These same studies suggest that BNDF is modifiable by metabolic markers (Levinger et al., 2008). It has been reported that obese people have lower BDNF levels compared to healthy controls (Lommatzsch et al., 2005); which was also observed in the current study. Based on this data, it can be assumed that some metabolic changes may impede the adaptation of BDNF to exercise (Levinger et al., 2008). Although studies have not been conducted on the effect of exercise

training on obese or overweight multiple sclerosis patients, previous studies on otherwise healthy obese individuals have shown that BDNF changes are closely associated with changes in body composition such as weight loss (Levinger et al., 2008). The lack of change in body composition in our study may provide a rationale as to why BDNF concentration was not impacted by the exercise intervention in obese multiple sclerosis participants.

The improvement observed in PDGF for both overweight and normal weight participants represents another beneficial effect of exercise training; as it has been shown that a higher level of PDGF can reduce clinical consequences of damage in multiple sclerosis and increase progenitors and reversal of cell death in CNS (Huang and Dreyfus, 2016). Exercise research on PDGF is limited, Czarkowska-Paczek et al., found that endurance training enhanced PDGF expression in rat skeletal muscle (Czarkowska-Paczek et al., 2010); however, Trenerry et al, reported although acute exercise increased PDGF expression, there was no change after 12 weeks of resistance exercise (Trenerry et al., 2011). The reason for the inconsistency of the results may be attributed to the type of exercise (endurance vs. resistance), the species (rat vs. human), and the assessment of tissue (serum vs. muscle tissue).

4.2. Exercise-Induced Alternation in BBB Permeability

A previous investigation on BBB in obese participants clearly indicated that BBB permeability was significantly higher than healthy control participants with normal weight (Roh et al., 2016). In line with previous studies (Roh et al., 2016), we showed that NSE was higher in overweight subjects at the baseline. A potential explanation for this issue is that obesity is associated with more reactive oxygen species (ROS) activity, which can be considered as a potential mechanism for greater BBB permeability (Roh et al., 2016). Additionally, the activity of pro-inflammatory cytokines and matrix metalloproteinases are known to play a crucial role in the function of the BBB (Rochfort et al., 2014; Zimmer et al., 2017). Therefore, the reduction in ROS and pro-inflammatory cytokines can be considered as strategies to maintain or restore BBB integrity. In these published studies, exercise was used as an effective strategy to modulate cytokines associated with BBB disruption (Rochfort et al., 2014; Roh et al., 2016; Zimmer et al., 2017). Our results also show that BBB permeability indicator reflected by s100b significantly

decreased after eight weeks of training. Bodyweight also seems to effect s100b concentrations in response to exercise training with s100b reduction significantly higher in NW-trained than other groups. In the current study, although cytokine levels did not move toward reducing inflammation, it can be deduced that by reducing BBB permeability, their progression and immune cells infiltration into the CNS has decreased, which can reduce CNS inflammation and lead to less neural damage (Raj et al., 2015). Moreover, previous data reported that PDGF can play an important role in the regulation of BBB permeability, and suggest potential strategies for treatment of non-multiple sclerosis patients (Raj et al., 2015). Therefore, greater increases of PDGF levels in the NW-trained group could be another reason for promote BBB permeability that was observed in the NW-trained group.

Importantly, a decrease in BBB permeability reduces the entry of glucocorticoids such as cortisol from the peripheral blood circulation to the CNS (Li et al., 2014). Cortisol is associated with greater numbers of active lesions and less remyelinated plaques. Therefore, less cortisol crossing the BBB promotes the possibility of remyelination and promoting immunodulatory function in the CNS (Moghadasi and Najafi, 2017).

Studies indicate that NSE concentration is indicative of brain and neural damage (Hoffmann et al., 2017; Marchi et al., 2004; Marchi et al., 2003; Roh et al., 2016). Furthermore, when there are both brain damage and BBB permeability, both s100b and NSE levels increase (Roh et al., 2016; Wu et al., 2016). Therefore, our results indicate that the effect of exercise in our subjects likely improved BBB permeability, but was not effective in reducing brain damage. As it is strongly evidenced that BBB destruction in multiple sclerosis patients is an introduction to brain damage (Cramer et al., 2014), reinforced BBB function may also be a viable mechanism demonstrating improvements in brain damage. Therefore, if the training period continues in patients, the results would be accompanied by a possible improvement in cerebral function markers. However, to reject or confirm this assumption, there is a need for further study with a longer training period.

4.3. Limitation

The current study has some limitation. Some of the evaluated markers in the current study are secreted from a variety of tissues. For example, BDNF is released from the brain, muscle, and some immune cells (Huang et al., 2014; Huang and Dreyfus, 2016; Onuoha et al., 1998). Considering that the evaluation of BDNF in the brain needs a cerebrospinal fluid sample or its evaluation in muscle requires biopsy. Collection of these bio specimens in multiple sclerosis patients can be painful; therefore, one of the limitations of this study is the lack of sampling of cerebrospinal fluid or muscle tissue.

Determining obesity can be done by a wide range of measures; BMI was used in the current study to determine weight status of participants. Although, BMI is one of the most common method to classification body type, it does not provide exact information on body composition (Frankenfield et al., 2001). Therefore, it is recommended to use further relatively straightforward methods (i.e. waist to hip circumference ratio) in combination with BMI in future studies to avoid this limitation.

There are several methods and techniques for determining the permeability of the BBB, which is a golden dynamic contrast-enhanced MRI (DCE-MRI) technique. Although the DCE-MRI has been used in numerous studies (Barnes et al., 2016; Côté et al., 2010; Montagne et al., 2015), it has been shown that peripheral markers (i.e. s100b) can also be suitable markers of BBB permeability (Hoffmann et al., 2017).

A final limitation is the lack of a healthy control group that could help to interpret the results. Moreover, the control condition did not receive any intervention, not even a minimal exercise treatment group using stretching and toning. This is important as the control condition only accounts for passage of time, maturation, and change in instrumentation, and does not account for social contact or attention. Future research should include a better control condition. Eventually, due to the nature of the current study design, the social status of the subjects could possibly influence our outcomes.

4.4. Conclusion

The current study indicates that 8 weeks of exercise training can stimulate BDNF and PDGF production and secretion in normal weight multiple sclerosis subjects. However, PDGF concentration increases were only observed in overweight multiple sclerosis subjects. Moreover, BBB permeability, as measured by s100b responded positively to exercise training in normal multiple sclerosis patients. Overall, exercise training can be an effective rehabilitation tool in people with multiple sclerosis, but current data demonstrated that the beneficial effects of exercise is restricted in overweight multiple sclerosis patients. Therefore, weight loss in overweight multiple sclerosis patients maybe important, and can be considered as the primary target of exercise training. Further comprehensive research is required to confirm the role of exercise on BBB permeability and examine the hypothesis that weight loss in people with multiple sclerosis can be considered as the primary target of exercise training.

Conflict of Interest statement

Mostafa Khodadust received support from Abadan Islamic Azad University, Abadan, Iran to perform current study. Other authors report no disclosures.

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Figures legend

Figure 1. Flowchart of experimental design and subjects included.

Figure 2. Effect of exercise training on neurotrophic factors and blood-brain barriers permeability.

* refers to significant difference between groups. NW-trained: normal training group, NW-control: normal control group, OW-trined: overweight training group, OW-control: overweight control group, BDNF: Brain-derived neurotrophic factor, NGF: Nerve growth factor, CNTF: Ciliary neurotrophic factor, PDGF: Platelet-derived growth factor, S100b: S100 calcium-binding protein B, NSE: neuron-specific enolase.

Figure 3. Effect of exercise training on cytokine profile and cortisol concentration.

NW-trained: normal training group, NW-control: normal control group, OW-trined: overweight training group, OW-control: overweight control group, TNF-a: Tumor necrosis factor alpha, IL-10: Interleukin 10.

Figure 4. Effect of exercise training on Vo_{2peak} and body composition.

NW-trained: normal training group, NW-control: normal control group, OW-trined: overweight training group, OW-control: overweight control group, BMI: body mass index, Vo_{2peak}: peak oxygen consumption.